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Stimulation and Growth of Antral Ovarian Follicles by Selective LH Activity Administration in Women

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Intensive FSH stimulation is a key tool of assisted reproduction technology but can cause severe complications through the development of an excessive number of small ovarian follicles. We tested the hypothesis that, in the late stages of ovulation induction, LH activity in the form of low-dose human CG (hCG) can stimulate and selectively modulate ovarian follicle function and growth, independently of FSH administration. Four groups of GnRH agonist-suppressed normoovulatory women (10 each group) received recombinant human FSH (r-hFSH) (150 IU/d) for 7 d followed by: group A, r-hFSH 150 IU/d alone; group B, r-hFSH 50 IU/d and hCG 50 IU/d; group C, r-hFSH 25 IU/d and hCG 100 IU/d; group D, hCG 200 IU/d alone. Despite several days of lowered or absent r-hFSH administration, 70% of hCG-treated patients successfully completed treatment. In these subjects, preovulatory E2 levels and large (>14 mm diameter) ovarian follicle development were not reduced; conversely, the number of small (<10 mm diameter) ovarian follicles was significantly decreased in groups B–D *vs.* group A. Low-dose hCG administration did not cause follicle luteinization. We conclude that, following FSH priming, LH activity administration can: 1) stimulate folliculogenesis for several days, in spite of rapidly declining FSH levels; and 2) hasten small follicle demise. Therefore, LH activity administration could be used to design radically novel ovulation induction regimens that, by partly or completely replacing mid-/late follicular phase FSH administration, may reduce costs and improve safety of assisted reproduction technology. (*J Clin Endocrinol Metab* 87: 1156–1161, 2002)

CONTROLLED OVARIAN STIMULATION (COS) is a critical component of assisted reproduction technology (ART), consisting of the administration of pharmacological doses of exogenous gonadotropins to achieve the development of multiple ovarian follicles and oocytes. Gonadotropin preparations used in COS contain FSH alone or in combination with variable amounts of LH activity (1). While FSH is considered the fundamental driver of folliculogenesis, the role of LH in this process is more controversial (2, 3). During the normal menstrual cycle, elevated FSH levels in the early follicular phase stimulate recruitment and growth of preantral and small antral follicles; in the mid- and late follicular phase, however, the decline of FSH concentrations and a progressive rise of LH levels are associated with the selection and growth of the dominant follicle destined for ovulation, and the demise of cohorts of smaller follicles. These events appear to be related to the expression of LH receptors on the granulosa cells (GC) of the dominant follicle that render it responsive to LH stimulation and lower dependence on FSH (4, 5).

Exogenous gonadotropin administration in COS causes a rise of FSH concentrations throughout the follicular phase; as endogenous LH secretion is usually suppressed with GnRH analogs and exogenous LH activity supplementation is either not provided (as with recombinant human FSH, r-hFSH) or modest (as with human menopausal gonadotropin, hMG), folliculogenesis in COS is mostly driven by FSH. The physiologic control mechanisms of the normal menstrual cycle are thus overridden, and the net result is the development of numerous ovarian follicles of all sizes. Although multiple folliculogenesis is the goal of COS, excessive follicle development is also the key factor involved in the pathogenesis of severe ovulation induction complications such as high order multiple gestations and the ovarian hyperstimulation syndrome (OHSS). In this study, we tested the hypothesis that LH activity, administered as low-dose hCG, can be used to stimulate the growth of large follicles and hasten the demise of small follicles when administered in the mid-/late follicular phase of COS treatment for ART. The application of the concepts derived from this study could be used to design ART ovulation regimens that combine efficacy with lower drug-related costs and increased safety through the potential reduction of complications.

Abbreviations: ART, Assisted reproduction technology; COS, controlled ovulation stimulation; CV, coefficient of variation; GC, granulosa cells; hCG, human CG; hMG, human menopausal gonadotropin; MDL, minimum detectable level; OHSS, ovarian hyperstimulation syndrome; P, progesterone; r-hFSH, recombinant human FSH.

Materials and Methods

Patient population

A total of 40 female patients diagnosed as having unexplained or mild male related infertility were studied. All subjects had regular menstrual cycles of 26–34 d duration, a normal body mass index of 20–25 kg/m^2, a pelvic ultrasound showing uterus and ovaries of normal size and structure, a hysterosalpingogram and/or laparoscopy demonstrating tubal patency, normal plasma and urinary chemistry and hematological values, thyroid and reproductive hormones within the normal range. Although ovulation induction had been previously performed in some of the subjects, no patient had received any hormone therapy (including gonadotropins) for at least 3 months preceding the study.

Protocol

Our Institutional Review Board approved the protocol and all patients provided informed consent. Patients underwent early follicular phase reproductive hormone determinations. The incidence of patients

who had previously undergone gonadotropin ovulation induction as well as cause of infertility were similar in all groups. Patients were not blinded to treatment, which was started in the mid-luteal phase of a spontaneous menstrual cycle with the administration of a single im injection of 3.75 mg of depot triptorelin (Decapeptyl IM, IPSEN S.p.A., Milan, Italy). Ovulation induction began 14 d thereafter. All patients menstruated before the initiation of gonadotropin treatment and pelvic ultrasound showed no formation of ovarian cysts after GnRH agonist administration. All patients started gonadotropin treatment with 150 IU (2 Amps) of sc r-hFSH daily (Puregon, Organon Italia S.p.A., Rome, Italy). On d 8 of treatment, patients were randomly assigned to 4 different age and weight-matched treatment groups, each comprising 10 subjects; group assignment was not in any way influenced by treatment outcome on d 1–7. The treatment regimens were the following: group A: r-hFSH, 150 IU, sc, daily, only; group B: r-hFSH, 50 IU, sc, and hCG (Profasi HP, Serono Pharma S.p.A., Rome, Italy) 50 IU, sc, daily; group C: r-hFSH, 25 IU, sc, and hCG 100 IU, sc, daily; group D: hCG, 200 IU, sc, daily, only (Table 1). In every patient, all gonadotropins were administered at 1600–1800 h and continued at the same dose until at least 4 ovarian follicles of >14 mm diameter and E2 levels of 800–1,500 pg/ml were detected (final maturation parameters). Treatment was discontinued in patients who menstruated during gonadotropin administration or did not achieve the final maturation parameters by the d 18 of gonadotropin administration. At the obtainment of the final maturation parameters, 10,000 IU of hCG were administered to trigger ovulation and intrauterine insemination with a sperm swim-up procedure was performed 36 h thereafter. The luteal phase was supported with 90 mg daily of intravaginal progesterone (P) gel (Crinone, Serono Pharma S.p.A., Rome, Italy) administered from d 3–14 following the preovulatory hCG dose.

Monitoring

Treatment monitoring was conducted throughout gonadotropin administration. Each day, one blood sample was drawn at 0800–0900 h in a standard manner, and two serum aliquots were obtained: E2 was measured daily in one of the serum aliquots for clinical monitoring, while the second aliquot was stored at −20 C for later measurements of LH, FSH, E2, P, T, and hCG. Transvaginal pelvic ultrasound was performed on menotropin treatment d 0 and 6 and at alternate days thereafter, until preovulatory hCG administration. The physician performing pelvic ultrasound was blinded as to which arm of the protocol each patient belonged.

Hormone assays

LH, FSH, E2, P, T, and hCG were measured with chemiluminescence assays (ADVIA ACS: Centaur, Bayer Corp. Diagnostics Division, Tarrytown, NY). The minimal detectable level (MDL) of LH was 0.1 IU/liter; the interassay coefficient of variation (CV) ranged between 3.9–6.0% at different levels of the standard curve. The *in vitro* addition of up to 200,000 IU/liter of hCG did not affect LH determinations in this assay, as assessed at multiple levels of the standard curve. The MDL of FSH was 0.3 IU/liter; the interassay CV ranged between 3.0–5.0% at different levels of the standard curve. The MDL of E2 was 10 pg/ml; the interassay CV ranged between 6.2–6.9% at different levels of the standard curve. The MDL of P was 0.1 ng/ml; the interassay CV ranged between 4.9–10.0% at different levels of the standard curve. The MDL of T was 0.1 ng/ml; the interassay CV ranged between 4.9–7.9% at different levels of the standard curve. The MDL of hCG in this β-specific assay was 0.1 IU/liter; the interassay CV ranged between 4.2–5.6% at different levels of the standard curve. The *in vitro* addition of up to 200 IU/liter of LH did not affect hCG determinations in this assay, as assessed at multiple levels of the standard curve.

TABLE 1. Regimens of gonadotropin administration

		Day 1–7	Day 8 to day of ovulation trigger
Group A	Daily r-hFSH dose (IU)	150	150
	Daily hCG dose (IU)	0	0
Group B	Daily r-hFSH dose (IU)	150	50
	Daily hCG dose (IU)	0	50
Group C	Daily r-hFSH dose (IU)	150	25
	Daily hCG dose (IU)	0	100
Group D	Daily r-hFSH dose (IU)	150	0
	Daily hCG dose (IU)	0	200

Statistical evaluation

Data were expressed as mean ± SEM. Serum hormone levels were calculated in each cycle as area under the curve. Among-group differences of continuous variables were assessed with Kruskal-Wallis one way ANOVA on ranks and the pairwise multiple comparison procedures (Tukey test). Nonparametric statistics (Mann-Whitney rank sum test) were used when only two groups of data were compared.

Results

The patient characteristics of the four treatment groups are shown in Table 2. No significant between-groups differences existed in age, height, weight, body mass index, pretreatment menstrual cycle duration, ovarian volume, and baseline hormone levels. All the patients of group A completed the treatment schedule while a total of 9 subjects treated with the combined r-hFSH and hCG regimens (3 in each group) did not achieve the final maturation parameters (P NS). Four conceptions were obtained, 2 in group A (1 singleton and 1 triplet), 1 in group B (singleton), 1 in group C (triplet); no miscarriages were reported. None of the treatment cycles resulted in moderate or severe OHSS.

The results of cycles outcome are presented using an intent to treat paradigm, *i.e.* primary outcomes assessment (except for the preovulatory follicle pattern and E2 concentrations) included all patients treated regardless of their achievement of the final maturation parameters. The clinical and endocrine results of treatment are shown in Table 3, and in Figs. 1 and 2. Duration of treatment and follicular phase LH, FSH, and E2 concentrations did not significantly differ among the 4 groups. The total r-hFSH and hCG doses employed were respectively lowest and highest in group D ($P < 0.001$). Follicular phase hCG levels were highest in group D ($P < 0.005$). Follicular phase P and T were lower in group A than in groups B and C ($P < 0.01$), and group B ($P < 0.05$), respectively. Preovulatory E2 levels did not differ in the 4 treatment groups among patients who achieved the final maturation parameters (Table 3).

Treatment discontinuation in patients who menstruated or did not achieve the final maturation parameters occurred between d 10 and 16. The E2 area under the curve in the initial 7 treatment days was 595 ± 100 pg/ml·d in patients who completed treatment (n = 31) and 227 ± 63 pg/ml·d in patients who did not achieve the final maturation parameters (n = 9) ($P < 0.05$). The number of ovarian follicles >10 mm diameter (intermediate and large) on d 8 of treatment was 4.1 ± 0.4 in patients who completed treatment and 2.1 ± 0.8 in patients who did not achieve the final maturation parameters ($P < 0.05$).

The pattern of ultrasound-detected ovarian follicles across treatment in all the patients treated (regardless of their achievement of the final maturation parameters) is shown in Fig. 3. The follicle pattern just before ovulation in the patients who achieved the final maturation parameters is shown in Fig. 4. While the number of large (diameter >14 mm) and

TABLE 2. Baseline parameters in the four treatment groups

	Group A	Group B	Group C	Group D	*P*
Baseline parameters					
Age (yr)	33.1 ± 0.9	33.1 ± 1.0	32.6 ± 1.1	30.4 ± 1.2	NS
Height (cm)	165 ± 1	170 ± 2	165 ± 2	165 ± 2	NS
Weight (kg)	58 ± 1	61 ± 1	57 ± 2	58 ± 2	NS
BMI (kg/m^2)	21.1 ± 0.3	21.2 ± 0.2	20.9 ± 0.2	21.3 ± 0.3	NS
Menstrual cycle duration (d)	27.9 ± 0.5	28.0 ± 0.5	27.9 ± 0.3	27.3 ± 0.5	NS
Mean ovarian volume (ml)	6.7 ± 0.4	6.4 ± 0.4	6.5 ± 0.3	6.4 ± 0.3	NS
LH (IU/liter)	4.7 ± 0.4	5.0 ± 0.6	4.4 ± 0.4	4.7 ± 0.5	NS
FSH (IU/liter)	6.5 ± 0.4	6.1 ± 0.6	5.8 ± 0.5	5.8 ± 0.5	NS
PRL (ng/ml)	16 ± 1	13 ± 2	14 ± 1	16 ± 3	NS
E2 (pg/ml)	79 ± 7	61 ± 8	67 ± 8	74 ± 9	NS
P (ng/ml)	0.56 ± 0.04	0.49 ± 0.07	0.52 ± 0.04	0.58 ± 0.06	NS
T (ng/ml)	0.48 ± 0.05	0.42 ± 0.07	0.39 ± 0.04	0.40 ± 0.06	NS

TABLE 3. Clinical and hormonal results of gonadotropin administration in the four treatment groups

	Group A	Group B	Group C	Group D	*P*
Daily r-hFSH dose (IU), d 8 onward	150	50	25	0	
Daily hCG dose (IU), d 8 onward	0	50	100	200	
Results of gonadotropin administration					
Days of gonadotropin administration	13.7 ± 1.0 (11–19)	13.4 ± 0.7 (10–16)	13.1 ± 0.6 (11–17)	12.7 ± 0.6 (10–15)	NS
Total r-hFSH dose received (IU)	1,920 ± 146[a]	1,325 ± 40[a]	1,180 ± 15[a]	1,050 ± 0[a]	<0.001
Total hCG dose received (IU)	0	275 ± 40[b]	520 ± 59[b]	940 ± 112[b]	<0.001
Preovulatory E2 (pg/ml)	1,034 ± 51	1,274 ± 113	1,223 ± 106	1,271 ± 105	NS
Follicular phase hormone levels					
LH (IU/liter·d)	12.0 ± 3.3	12.3 ± 1.3	13.8 ± 1.0	12.4 ± 1.7	NS
FSH (IU/liter·d)	96.6 ± 12.3	91.2 ± 3.4	79.8 ± 7.0	80.7 ± 5.5	NS
hCG (IU/liter·d)	ND	10.2 ± 3.0[b]	11.8 ± 2.8[b]	38.5 ± 8.6[b]	<0.005
E2 (pg/ml·d)	3,651 ± 466	3,695 ± 662	3,929 ± 798	3,902 ± 677	NS
P (ng/ml·d)	7.4 ± 1.0[c]	10.7 ± 0.8[c]	10.7 ± 0.8[c]	8.1 ± 0.7	<0.01
T (ng/ml·d)	4.2 ± 0.6[d]	6.8 ± 0.6[d]	4.9 ± 0.4	4.9 ± 0.7	<0.05

[a] $P < 0.05$ group A *vs.* groups C & D, group B *vs.* group D.
[b] $P < 0.05$ group D *vs.* groups B & C.
[c] $P < 0.05$ group A *vs.* groups B & C.
[d] $P < 0.05$ group A *vs.* group B.

intermediate (10–14 mm) preovulatory follicles did not significantly differ among the 4 groups, the number of small preovulatory follicles was significantly higher in group A (8.1 ± 0.5) than in the other treatment groups (B 3.1 ± 1.6, C 3.0 ± 1.3, D 1.9 ± 0.7; $P < 0.001$).

Discussion

LH is a critical component of follicular phase physiology, often overlooked in ovulation induction (2). In addition to promoting theca cells androgen substrate production for estrogen synthesis, LH can stimulate GC function, once ovarian follicles grow roughly beyond a 10-mm diameter size; this feature is due to FSH- and estrogen-induced LH receptor expression by GC (6, 7). In the normal menstrual cycle, continued dominant follicle growth in spite of declining FSH levels is likely brought about by LH stimulation of GC that have acquired LH receptors (8). It was demonstrated that LH activity in the form of r-hLH (9) or low-dose hCG (10) is capable of directly stimulating large ovarian follicle development in the presence of fixed or increasing FSH levels. In the current study, we wanted to test the ground-breaking hypothesis that, once FSH-induced follicle recruitment is achieved in COS, the administration of LH activity can partly or completely replace FSH for the support and stimulation of larger follicle(s) growth, thus allowing for exogenous FSH dose reductions or discontinuation. We also reasoned that the lowering of FSH dose administration allowed by LH activity replacement could hasten the demise of smaller follicles that are devoid of GC LH receptors and whose support is fully dependent on FSH stimulation; the occurrence of small-size preovulatory ovarian follicles is directly related to the development of ovulation induction complications (11).

Following 7 d of a fixed dose of r-hFSH administration (150 IU daily), patients were randomized to receive different amounts of r-hFSH and/or hCG until proper large follicle number and E2 levels were achieved (Table 1). hCG selectively binds to LH receptors and exerts the same actions as LH (12); however, also because of its more prolonged half-life, hCG is approximately 6 times more potent than LH (13). We chose to use hCG instead of r-hLH because the longer decay period of hCG (14) ensured a more stable stimulation of LH receptors over the 24 h intervals between hormone administrations. We previously demonstrated that low-dose hCG (50 IU daily) and FSH activity safely and effectively synergize to more intensely stimulate large ovarian follicle development and reduce treatment duration and FSH dose requirements (10, 15).

Treatment was completed by all the patients who received r-hFSH only (group A) and in 7 out of 10 patients in each of the other 3 groups. Inspection of treatment cycles in the

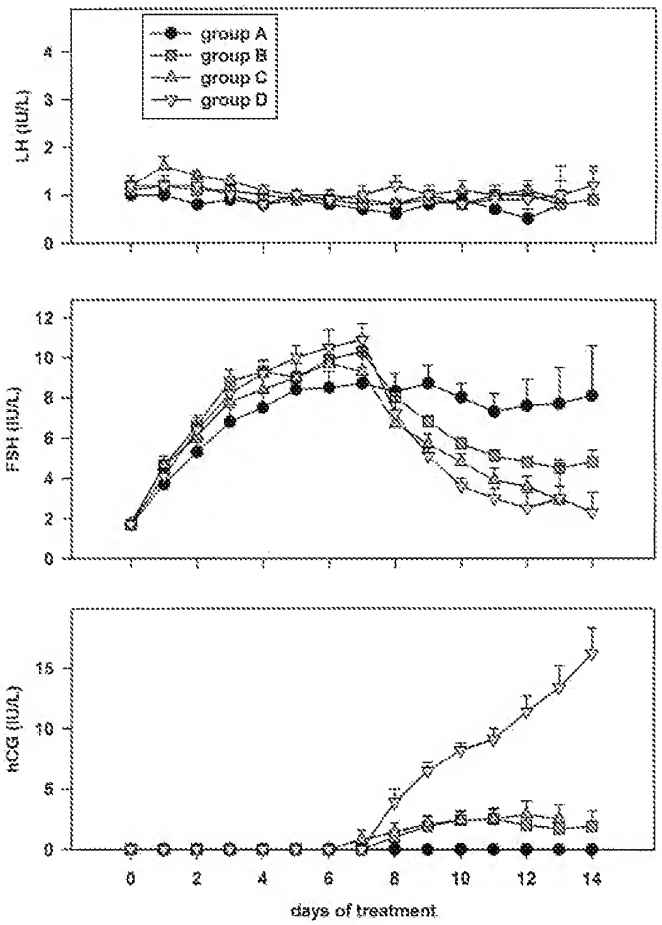

FIG. 1. Gonadotropin concentrations. Daily gonadotropin serum levels (mean ± SEM) in the 4 groups of patients participating in the study.

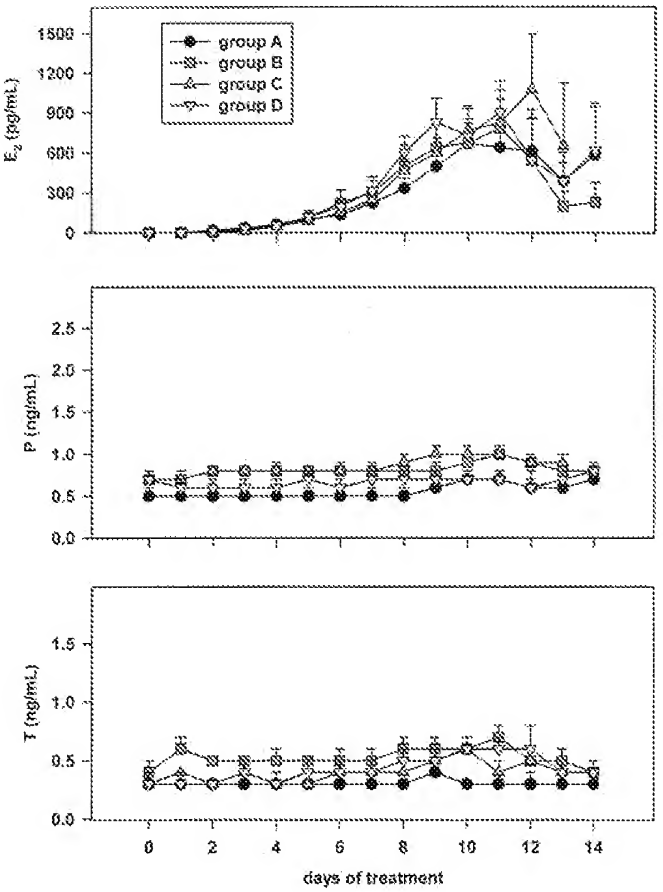

FIG. 2. Steroid concentrations. Daily gonadal steroid serum levels (mean ± SEM) in the 4 groups of patients participating in the study.

patients who did not attain the final maturation parameters revealed that lack of response following partial or complete replacement of r-hFSH with low-dose hCG was associated with the presence of fewer medium and large size ovarian follicles on d 8 of treatment and lower early/mid-follicular phase serum E2 levels. Our protocol did not allow for changes in the day when we switched the stimulation from r-hFSH only to r-hFSH/hCG or hCG alone; thus, it is likely that by treatment d 8 some patients may not have developed a sufficient number of mature follicles and/or proper amounts of granulosa cell LH receptors for the hCG support to be effective. The E2 and follicle patterns among patients who later failed to achieve the final maturation parameters appears to support this interpretation. Thus, when follicle development was quantitatively and/or qualitatively inadequate or delayed, LH activity administration in the presence of declining FSH levels appeared to be incapable of sustaining folliculogenesis. Conversely, in patients who later attained final maturation parameters, no significant difference existed in the number of large and intermediate preovulatory follicles (Fig. 4) and in preovulatory E2 concentrations (Table 3) among the four groups; the amount of r-hFSH needed to achieve the final maturation parameters decreased progressively from group A to group D, as the amount of exogenous hCG employed rose (Table 3). The negative treatment outcome we saw in 9 out of 30 hCG-treated patients suggests that combined FSH and low-dose hCG regimens may need to be further refined to better identify patients who have developed LH-responsive follicles. Nevertheless, the key finding of our study was that in most gonadotropin-induced cycles LH activity alone (or combined with much reduced exogenous FSH administration) could successfully complete COS in FSH-primed patients.

Hormone secretion pattern inspection showed no significant differences in the serum levels of LH (Fig. 1) and E2 (Fig. 2) among the four treatment groups; conversely, as expected by the regimens employed, post treatment d 7 serum levels of FSH and hCG respectively declined and increased from group A to group D (Fig. 1). None of these hormone patterns negatively affected the development of large and medium ovarian follicles (Fig. 3); conversely, the number of small (<10 mm) preovulatory follicles was significantly reduced in all the patients receiving exogenous hCG (Fig. 4), similar to what we recently showed in patients receiving hMG (16). These findings are consistent with the concept that LH is capable of selectively promoting larger follicle growth, as we know that simple suspensions or dose reductions of FSH administration in the mid-/late follicular phase (without LH activity supplementation) are associated with an arrest or a severe curtailment of folliculogenesis and declining serum E2 concentrations (17, 18); rescue of larger follicles (>10 mm)

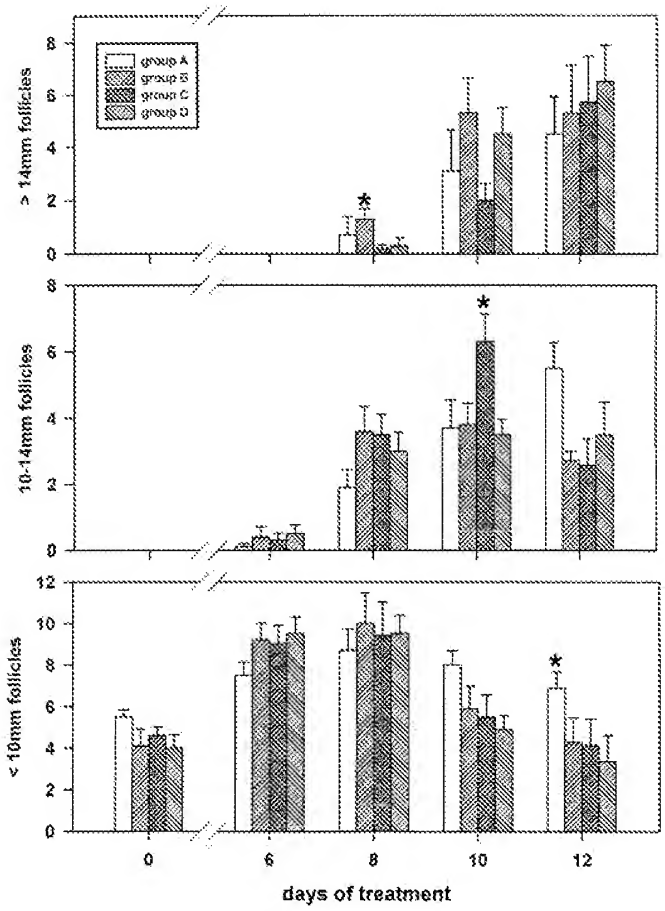

FIG. 3. Patterns of follicle development. Number (mean ± SEM) of small (<10 mm diameter), medium (10–14 mm), and large ovarian follicles (>14 mm) measured with transvaginal pelvic ultrasound throughout gonadotropin administration in treatment groups A–D. *Asterisks* (*) indicate significant differences ($P < 0.05$) in the number of follicles in one of the treatment groups on one specific treatment day.

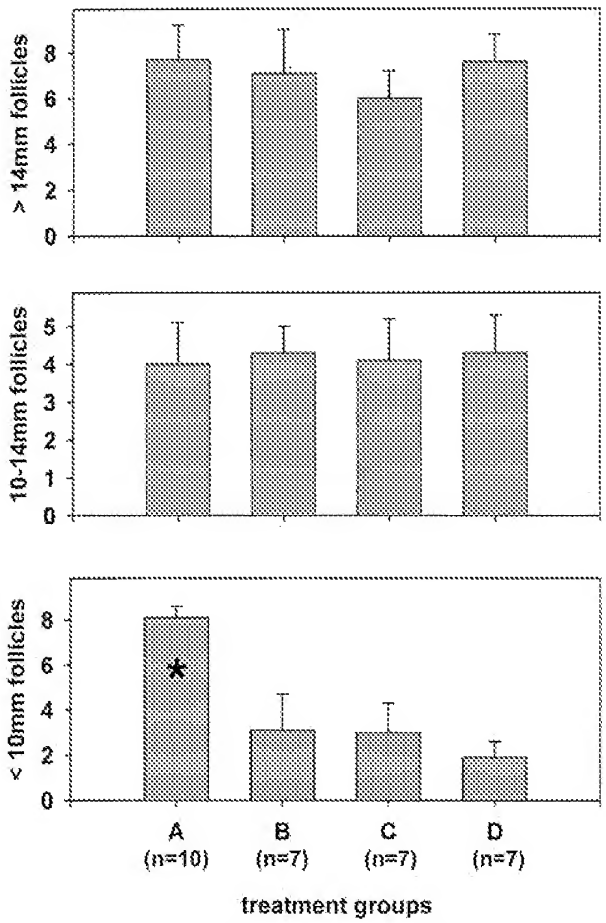

FIG. 4. Preovulatory follicles. Number (mean ± SEM) of preovulatory small (<10 mm diameter), medium (10–14 mm), and large ovarian follicles (>14 mm) measured with transvaginal pelvic ultrasound on the last day of gonadotropin administration. *, $P < 0.001$.

was likely due to the expression of GC LH receptors that rendered them exquisitely receptive to LH stimulation. Conversely, selective regression of smaller ovarian follicles could be related to reduced FSH support and/or to increments of intrafollicular androgen concentrations associated with increased LH stimulation (19).

The possibility of modulating follicle function and development with exogenous LH activity with or without reduced FSH stimulation was previously theorized (5, 20) and tested in protocols that employed r-hLH. Sullivan *et al.* (18) replaced r-hFSH with r-hLH only in the last 2 d of ovarian stimulation of r-hFSH primed patients candidates for ART; although they provided no information regarding the pattern of ovarian follicle development, adequate serum E2 levels were maintained by r-hLH administration alone. Preliminary data reported in hypogonadotropic and polycystic ovary syndrome patients (21, 22) treated with r-hFSH for non-ART ovulation induction also suggested that r-hLH can hasten small ovarian follicle demise and permit to selectively achieve monofolliculogenesis, thus reducing the potential risk of ovulation induction-related complication. Our study for the first time demonstrated that intense LH activity stimulation over several days of treatment, even in the absence of FSH administration, can result in COS optimization by providing support and stimulation of larger ovarian follicles development while at the same time hastening small follicles demise. We also confirmed (10, 15) that low-dose hCG (which is also contained in hMG) (16) can represent an adequate source of selective LH activity in ovulation induction protocols; the lengthened half-life of hCG is an attractive feature of this compound as it can provide a more prolonged and stable stimulation of LH receptors than r-hLH in-between daily hormone administrations. An additional interesting feature of our study was that the increase of serum P and T concentrations following 50–200 IU of hCG was barely significant and limited to only some of the patients (Fig. 2 and Table 3). These modest increments of gonadal steroids following the administration of much greater hCG amounts than previously reported (23) challenges the concept that moderate follicular phase increments of LH and/or hCG can cause premature follicle luteinization, endometrial derangements responsible for embryo implantation disorders, or other untoward effects (24, 25).

In summary, this study provided novel information on COS and ovulation induction regimens in general. Although the role of LH was for a long time underestimated or even

considered detrimental (26), we demonstrated that the selective addition of LH activity in the form of low-dose hCG can be exploited to devise treatment regimens that, by improving folliculogenesis and reducing FSH dose requirements, could provide optimal treatment outcome and at the same time markedly reduce the cost of ovulation induction procedures. Furthermore, our results suggest that the use of LH activity administration in the late ovulation induction stages can selectively reduce the occurrence of small preovulatory ovarian follicles and, potentially, of OHSS (11), thus improving the safety of ART procedures. Although we cannot exclude that low levels of FSH activity may still be needed to optimize the late folliculogenesis stages, novel and unconventional protocols could be envisioned, consisting of initial higher dose FSH administration to boost follicle recruitment, followed (once the desired number of medium size follicles has emerged) by FSH curtailment or discontinuation and LH activity administration until ovulation to selectively promote larger follicle development (1). Such an approach may profoundly modify the current management of anovulation and ART. Additional investigations will be needed to further assess the specific effects of r-hLH and low-dose hCG administration in different clinical conditions and regimens.

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References

1. **Filicori M, Cognigni GE** 2001 Roles and novel regimens of luteinizing hormone and follicle stimulating hormone in ovulation induction. J Clin Endocrinol Metab 86:1437–1441
2. **Filicori M** 1999 The role of luteinizing hormone in folliculogenesis and ovulation induction. Fertil Steril 71:405–414
3. **Levy DP, Navarro JM, Schattman GL, Davis OK, Rosenwaks Z** 2000 The role of LH in ovarian stimulation: exogenous LH: let's design the future. Hum Reprod 15:2258–2265
4. **Hillier SG, Whitelaw PF, Smyth CD** 1994 Follicular oestrogen synthesis: the 'two-cell, two-gonadotrophin' model revisited. Mol Cell Endocrinol 100:51–54
5. **Campbell BK, Dobson H, Baird DT, Scaramuzzi RJ** 1999 Examination of the relative role of FSH and LH in the mechanism of ovulatory follicle selection in sheep. J Reprod Fertil 117:355–367
6. **Zeleznik AJ, Midgley-AR J, Reichert LEJ** 1974 Granulosa cell maturation in the rat: increased binding of human chorionic gonadotropin following treatment with follicle-stimulating hormone *in vivo*. Endocrinology 95:818–825
7. **Shima K, Kitayama S, Nakano R** 1987 Gonadotropin binding sites in human ovarian follicles and corpora lutea during the menstrual cycle. Obstet Gynecol 69:800–806
8. **Zeleznik AJ, Hillier SG** 1984 The role of gonadotropins in the selection of the preovulatory follicle. Clin Obstet Gynecol 27:927–940
9. **The European Recombinant Human LH Study Group** 1998 Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. J Clin Endocrinol Metab 83:1507–1514
10. **Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, Tabarelli de Fatis C, Pocognoli P** 1999 Luteinizing hormone activity supplementation enhances follicle-stimulating hormone efficacy and improves ovulation induction outcome. J Clin Endocrinol Metab 84:2659–2663
11. **Navot D, Relou A, Birkenfeld A, Rabinowitz R, Brzezinski A, Margalioth EJ** 1988 Risk factors and prognostic variables in the ovarian hyperstimulation syndrome. Am J Obstet Gynecol 159:210–215
12. **Ross GT** 1977 Clinical relevance of research on the structure of human chorionic gonadotropin. Am J Obstet Gynecol 129:795–808
13. **Stokman PG, de Leeuw R, van den Wijngaard HA, Kloosterboer HJ, Vemer HM, Sanders AL** 1993 Human chorionic gonadotropin in commercial human menopausal gonadotropin preparations. Fertil Steril 60:175–178
14. **Damewood MD, Shen W, Zacur HA, Schlaff WD, Rock JA, Wallach EE** 1989 Disappearance of exogenously administered human chorionic gonadotropin. Fertil Steril 52:398–400
15. **Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, Tabarelli De Fatis C** 1999 Low-dose human chorionic gonadotropin therapy can improve sensitivity to exogenous follicle-stimulating hormone in patients with secondary amenorrhea. Fertil Steril 72:1118–1120
16. **Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, Tabarelli De Fatis C, Pocognoli P, Cantelli B, Boschi S** 2001 Luteinzing hormone activity in menotropins optimizes folliculogenesis and treatment in controlled ovarian stimulation. J Clin Endocrinol Metab 86:337–343
17. **Egbase PE, Al Sharhan M, Berlingieri P, Grudzinskas JG** 2000 Serum oestradiol and progesterone concentrations during prolonged coasting in 15 women at risk of ovarian hyperstimulation syndrome following ovarian stimulation for assisted reproduction treatment. Hum Reprod 15:2082–2086
18. **Sullivan MW, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ** 1999 Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab 84:228–232
19. **Filicori M, Flamigni C, Cognigni GE, Falbo A, Arnone R, Capelli M, Pavani A, Mandini M, Calderoni P, Brondelli L** 1996 Different gonadotropin and leuprorelin ovulation induction regimens markedly affect follicular fluid hormone levels and folliculogenesis. Fertil Steril 65:387–393
20. **Hillier SG, Smyth CD, Whitelaw PF, Miro F, Howles CM** 1995 Gonadotrophin control of follicular function. Horm Res 43:216–223
21. **Arguinzoniz M, Duerr-Myers L, Engrand P, Piazzi A, Loumaye E,** The efficacy and safety of recombinant human luteinizing hormone for minimizing the number of pre-ovulatory follicles in WHO group I anovulatory women treated with rhFSH. Proc 16th Annual Meeting of the European Society of Human Reproduction and Embryology, Bologna, Italy, 2000, Hum Reprod (Abstract book 1), pp 70–71
22. **Loumaye E, Duerr-Myers L, Engrand P, Piazzi A, Arguinzoniz M,** Minimizing the number of pre-ovulatory follicles in WHO group II anovulatory women over-responding to FSH with recombinant human luteinizing hormone. Proc 16th Annual Meeting of the European Society of Human Reproduction and Embryology, Bologna, Italy, 2000, Hum Reprod (Abstract book 1), p 71
23. **Copperman AB, Horowitz GM, Kaplan P, Scott RT, Navot D, Hofmann GE** 1995 Relationship between circulating human chorionic gonadotropin levels and premature luteinization in cycles of controlled ovarian hyperstimulation. Fertil Steril 63:1267–1271
24. **Chappel SC, Howles C** 1991 Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. Hum Reprod 6:1206–1212
25. **Hillier SG** 2000 The Parkes lecture: controlled ovarian stimulation in women. J Reprod Fertil 120:201–210
26. **Daya S, Gunby J, Hughes EG, Collins JA, Sagle MA** 1995 Follicle-stimulating hormone versus human menopausal gonadotropin for *in vitro* fertilization cycles: a meta-analysis. Fertil Steril 64:347–354